

## **In Vivo Administration of AAV Vector to Mouse by Tail Vein Injection**

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### 1. INTRODUCTION

This protocol is a general summary of many protocols in the literature that aim to deliver AAV particles systemically to mouse via intravenous injection. In vivo application of AAV vectors is a complex issue. The experimental design varies according to the specific goals that researchers want to achieve. This protocol could serve as a starting point or a reference to help investigators to design their own.

### 2. MATERIAL

#### 2.1 AAV vectors

Sterile filtered, purified AAV vectors at 1e12 genome copy (GC)/ml produced at Applied Viromics (or vectors with comparable quality).

Vector needs to be purified to homogeneity (free from HEK 293 proteins, 293 genomic DNA or plasmid DNA, endotoxin, or serum component). The viral titer ought to be high enough to allow sufficient dose to be delivered in small volume (see below).

Vector can be formulated in PBS, in basal cell culture medium, or other buffer systems (Tris, for example) with or without supplement (sugar, surfactant, etc.). AAV vectors from Applied Viromics are formulated in PBS, pH 7.4 with 0.001% Pluronic F-68.

#### 2.2 Mouse strain

C57BL/6 or BALB/c mice are commonly used. However, one can choose immunodeficient strains such as nude, scid, or Rag1 (Jackson Laboratory, Bar Harbour, Maine, USA) if neutralizing antibody against transgene products or AAV particles is a concern.

6 week old mice are preferred for infection study, unless the research design requires otherwise.

#### 2.3 Animal facility

Such a facility is required for the purpose of maintaining the mice, injection operation, and sampling.

### 3. METHOD

#### 3.1 Experimental design

It is recommended that the mice be treated in three groups:

Group1 – mock infection. Excipient only will be injected (the same volume as viral vectors).  
 Group2 – control vector infection. AAV with marker genes (such as GFP or lacZ) be injected (same titer).  
 Group3 – test vector infection. Multiple doses are recommended (see below).

Control vector and test vector need to be produced and assayed with the same methods to eliminate vector quality issues and titer discrepancies. Applied Viromics offer pre-made AAV control vectors to investigators who order custom-produced vectors for animal studies.

### 3.2 Dose design

It is recommended that three doses of AAV vector be administered as below:

	Low dose	Mid dose	High dose
(GC/mouse)	3e10	1e11	3e11
(GC/kg body weight)	1.2e12	4e12	1.2e13
Animal number	3 - 6	3 - 6	3 - 6

It is up to the investigator to judge whether it is necessary to have all three doses for the control.

Depending on the readout of the experiment, more mice per group are needed if mice will be sacrificed as the way of data acquisition during the course of the study (see section 3.4).

### 3.3 Tail vein injection

- 3.3.1 Appropriately mark mice used in the experiment.
- 3.3.2 Prepare dilutions of AAV vectors (both control and test vectors) so that the amount of genome copy listed in the table above can be delivered in 200µl volume. Use the same volume of excipient as mock infection.
- 3.3.3 Warm the tail by immersing it in warm water or placing the animal under a heat lamp. The tail vein is easier to see in non-pigmented mice. Perform the injection using fine gauge needle (26-30), and finish injection of 200µl volume within 15sec.

### 3.4 Data acquisition

The following timetable is recommended for data collection.

Immediately before infection (as base line);  
 Weekly from start to 1 month;  
 Bi-weekly up to 3 month;  
 Monthly afterwards.

One can start sampling much earlier (hours after injection) if issues such as viral distribution/clearance are the topics of study. More time points can be used if sampling only involves non-surgical procedures such as taking blood for assay or other non-invasive testing. Significant more mice need to be infected (see section 3.2) when mice will be sacrificed at each time points.

## 4. RESULTS

### 4.1 Time and duration of transgene expression

The on-set of transgene expression will start 1 week post-infection. This time is affected by many factors, such as promoter type, the nature of transgene, expression level of the encoding protein as well as assay sensitivity.

Transgene expression from AAV infection can be long term. Reports for more than 2 years of continuous expression are available in the literature.

### 4.2 Transgene expression levels

One may see a transient phase of transgene expression in the first 1-2 days. This is from the large amount of un-incorporated AAV genome that gets quickly cleared from the infected cells. After this expression spike, gene expression level will gradually increase over time and either maintain or settle at a stable level for extended period of time.

The level of transgene expression in general correlates with the dose of AAV administered. However, a maximum level can be reached with high enough dose. The status of neutralizing antibody (both pre-existing and induced) plays a significant role in controlling the gene expression levels in the animal study. A determination of neutralizing antibody titer is highly recommended.

### 4.3 Viral distribution

Intravenous injection of AAV leads the vector to almost all the organs of the body, especially cumulating in the liver, spleen, lung, kidney, and vasculatures. The transgene expression profiles, however, will be determined by the promoter (whether tissue-specific), the gene itself, and the serotype of AAV.

## 5. NOTE

For questions about this protocol, please contact Applied Viromics, LLC at (510) 266-2646 or email [info@appliedviromics.com](mailto:info@appliedviromics.com).